Accompanying Documents

Accompanying this amendment are the following documents:

(1) Marked-up version of the claims, showing the amendments made herein;

(2) Currently pending claim set, incorporating the amendments made herein;

(3) Sequence Listing in computer-readable form (CRF) and paper copy;

(4) Statement declaring that contents of CRF and paper copies of the Sequence Listing are the same, as required by 37 C.F.R. 1.821(e);

(5) Copies of Form PTO-1449s sent with the Information Disclosure Statement submitted February 12, 2001 and the Supplemental Information Disclosure Statement submitted August 1, 2001.

AMENDMENT

In the Claims:

Please cancel claim 25, without prejudice and without disclaimer.

Please amend Claim 24 as follows:

24. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an

ecdysone receptor (EcR) and further comprises a coding sequence encoding a retinoid-X-receptor (RXR), wherein said EcR and RXR coding sequences are operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

Please add the following claims:

30. (New) The method of claim 24, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

31. (New) The method of claim 30, wherein the enhancer sequence is an SP1 enhancer sequence.

32. (New) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control

elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

33. (New) The method of claim 32, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

34. (New) The method of claim 33, wherein the enhancer sequence is an SP1 enhancer sequence.

~~35.~~ (New) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing a mammalian cell comprising a retinoid-X-receptor (RXR) with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

A2 cont'd

36. (New) The method of claim 35, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

37. (New) The method of claim 36, wherein the enhancer sequence is an SP1 enhancer sequence.--

In the Specification:

Please enter the accompanying initial paper copy of the "Sequence Listing" into the specification.

Please replace the paragraph beginning on page 30, line 7 with the following rewritten paragraph:

A4

p4.1c: A synthetic DNA encoding the restriction enzyme sites NotI-MluI-Ecl136II-SstII-SfuI-SmaI-SfuI-ClaI-BglII-SnaBI-BstEII-PmlI-RsrII-NotI and having the sequence (CGGCCGCACGCGTGAGCTCCGCGGTTCGAATCCCGGGATTCGAACAT CGATAAAAGATCTACGTAGGTAACCACGTGCGGACCGAGCGGCCGC) (SEQ ID NO:1) was cloned into the blunted KasI and EarI(partial) sites of pUC119 (the vector fragment is 2757bp in length). A 653bp SpeI(blunted)-SacII(blunted) fragment encoding the CMV immediate early (IE) promoter, and a 488bp, SmaI-DraIII fragment containing the human growth hormone polyadenylation site, were cloned into the Ecl136II and SnaBI sites of the afore-mentioned plasmid, respectively. A chimeric intron composed of the splice donor from the first intron of CMV IE gene and the splice acceptor from the second intron of the human ß- globin gene was then installed into the SmaI site of the plasmid in two steps. A DNA fragment encoding the CMV IE gene first intron splice donor was produced by PCR using isolated CMV DNA (strain ad169) as template and the following primers, GGCCGGGAACGGTGCATT (SEQ ID NO:2), and GGGCAAGGGGGTGGGCCTATA (SEQ ID NO:3). This 87 bp

A 4 cont'd

fragment was ligated into the SmaI site of the plasmid intermediate. The resulting plasmid was cleaved with BstXI and SmaI, blunted with T4 DNA polymerase, and a 398bp DraI-EcoRI(blunt) fragment encoding the human ß-globin second intron splice acceptor was ligated into the plasmid. The construction of p4.1c was completed by ligation of a polylinker encoding the restriction sites ClaI-EcoRI-SmaI-BamHI-XbaI-SalI-PstI-HinDIII-XhoI-Eco47III-XhoI-BglII between the ClaI and BglII sites of the last intermediate plasmid. The sequence of this synthetic DNA was
ATCGATTGAATTCCCCGGGGATCCTCTAGAGTCGACCTGCAGAAGCTT
GCTCTCGAGCAGCGCTGCTCGAGAGATCT (SEQ ID NO:4).

Please replace the paragraph beginning on page 31, line 1 with the following rewritten paragraph:

A 5

p4.1c mEPO: p4.1c was digested with SmaI and a 2812bp SmaI(partial)-NcoI(blunted) fragment encoding all of the exons of the mouse erythropoietin gene was inserted. The Kozak sequence around the initiator methionine was changed to the optimally translated sequence, CCACCATG (SEQ ID NO:5), using oligonucleotide directed mutagenesis. The sequence of the mutagenic oligonucleotide was AGCTAGGCGCCACCATGGGGGTGC (SEQ ID NO:6).

Please replace the paragraph beginning on page 31, line 8 with the following rewritten paragraph:

A 6

pV4.1c mEPO: The polylinker and lacZ alpha fragment expression cassette of pUC119 was replaced by a single Sse8387I site by ligation of the following synthetic DNA fragment in the plasmid vector after digestion with AflIII and EheI, GGCGCCCCTGCAGGACATGT (SEQ ID NO:7). The resulting plasmid was cut with Sse8387I and the 4772bp Sse8387I fragment from pW1909adhlacZ that contains the ITR-bounded lacZ expression cassette was ligated to it. The resulting plasmid was called intermediate1. Next, p4.1c mEPO

A 4 cont'd

was digested with NotI and the 4582bp fragment encoding the mEPO expression cassette was isolated. One copy of a synthetic DNA fragment that encodes the D region of the AAV ITR was ligated to each end. The sequence of this synthetic fragment was GCGGCCGCAGGAACCCCTAGTGATGGAGTTGG (SEQ ID NO:8). The product of this reaction was ligated into the 2831bp, plasmid vector encoding MscI fragment of intermediate1(above) to form pV4.1c mEPO.

Please replace the paragraph beginning on page 31, line 21 with the following rewritten paragraph:

A 7

p4.1c hEPO: p4.1c was cleaved with SmaI and the 718bp, PpuMI-NcoI fragment of the human Epo cDNA (blunted) was ligated into this site. The translational initiation sequence was then modified by oligonucleotide -directed mutagenesis using the following mutagenic oligo: CATCGATTGAATTCCACCATGGGGGT (SEQ ID NO:9). The resulting construct was cleaved with Pml I and the 1765bp, EcoRV-HincII fragment of the LacZ gene was ligated into it.

Attached hereto is a marked-up version of the changes made to the claims and specification by the current amendment. The pages are captioned "**Version with markings to show changes made**."